Chapter 32 | Troubleshooting Procedure for **Artisan**Link Special Staining System

Debra Cobb, HT, MBA

Automation in histology is here to stay. With the shortage of experienced histotechnologists, an increased workload, and demands for faster turnaround times, laboratories are looking for ways to produce consistent, quality-stained slides with less staffing in the most efficient time.

The arrival of automated instruments has forced histotechnologists to familiarize themselves with hardware, software and chemistry in order to adequately troubleshoot automated platforms. Understanding the differences and the similarities between manual and automated special staining is half the battle when it comes to troubleshooting.

Back to Basics

Every histotechnologist understands that to achieve quality staining results performed manually the following basic factors should be in place in the laboratory:

1. Proper grossing, infiltration of tissue with fixative, and dehydration steps.
2. A certain tissue thickness recommended for a particular procedure. For instance, it is recommended to cut tissue for staining with Congo Red into a thickness of 8 µm and tissue for staining with Jones' Basement Membrane Stain is recommended to be cut into a thickness of 2 µm.
3. Drying slides at the right temperature because drying slides at temperatures above 62° C can cause damage to the tissue leading to a suboptimal staining.

The same basic factors are required for staining slides on an automated instrument such as **Artisan**Link. However, two more factors should be taken into consideration:

(a) The user must follow the procedures and instructions as provided by the instrument manufacturer.

(b) The user must follow a daily maintenance procedure in addition to the biannual or annual preventive maintenance procedure.

Troubleshooting Issues

When troubleshooting **Artisan**Link Special Staining System, the histotechnologist will not only be required to rely on his/her knowledge of histology procedures and special stains chemistry, but also on his/her computer skills in order to optimize staining protocols using the **Artisan**Link software. Because the instrument applies reagents as well as heats and incubates according to the instructions given by the user, it is important that the user programs the instrument properly taking into consideration that all other processes such as grossing, sectioning, fixation, etc are optimized before the sample is placed on the instrument. In other words, how to optimize individual slides based on prestaining processes can only be defined by a user.

The most common staining issues on **Artisan**Link are:

- Staining artifacts
- Background staining
- Inconsistent staining

Staining Artifacts

Problem:

(1) Tissue slides stored for long periods of time (months to years).

(2) Dust particles settling on slides, especially, those that are charged.

(3) Dispensing of reagents from dirty bulk liquid containers (Fig. 1).

Solution:

(1) For slides that may be dusty, it is recommended to try the following procedure after the deparaffinization and hydration steps:

- Place the slides in running tap water for 2-3 minutes.
- Rinse in deionized water.
- Place the slides in Artisan™ Wash Solution for 5 minutes before loading them on **Artisan**Link.

(2) For slides that have dispensing artifacts, it is recommended to use clean bulk liquid containers.

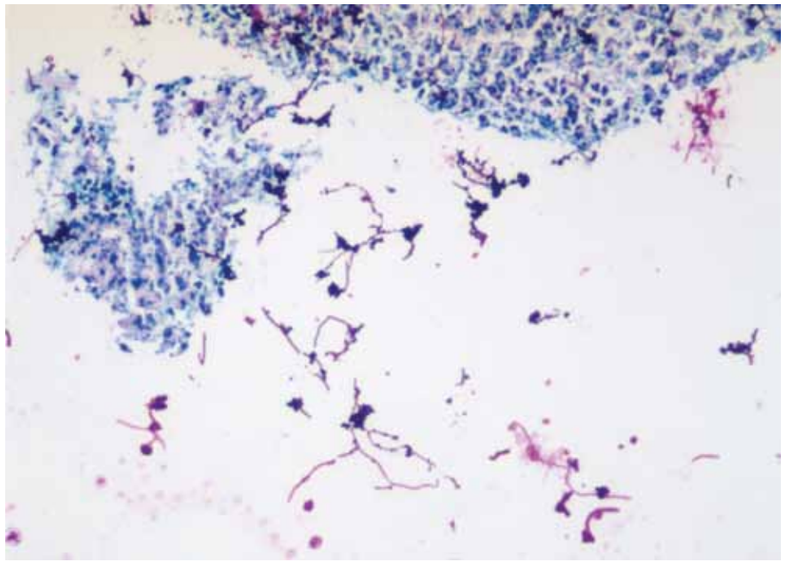

Figure 1. Artifacts on slides caused by dirty bulk liquid containers.

Reagent packs that are placed on the instrument need to be checked for any type of build-up of chemical deposits that can be dispensed on the slide during the staining run. It is recommended to check the dispenser tip and to mix and prime the packs before daily use (Fig. 2). It is also important to store the reagent packs tip down to prevent damage to the dispenser's internal parts.

Staining artifacts may also arise from reagent dyes. Dyes such as Nuclear Fast Red or Crystal Violet are known to cause artifacts. For instance, Nuclear Fast Red is sensitive to cold. If the reagent is stored in the refrigerator or left out on the loading dock in freezing weather, there is a risk that the reagent will start to precipitate. Once this reaction starts, it will continue regardless of correcting the storage temperature. This precipitate will then be dispensed onto the slide (Fig. 3a). Crystal Violet is another example of a dye that has a tendency to leave crystal-like shards on the tissue (Fig. 3b).

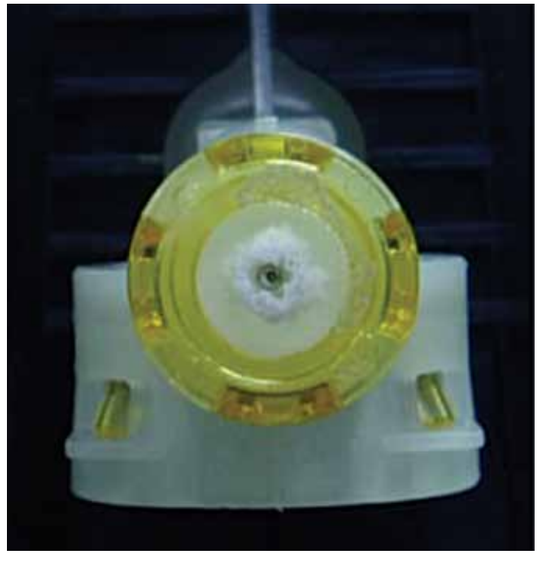

Figure 2. Dispenser showing build-up of chemical deposits.

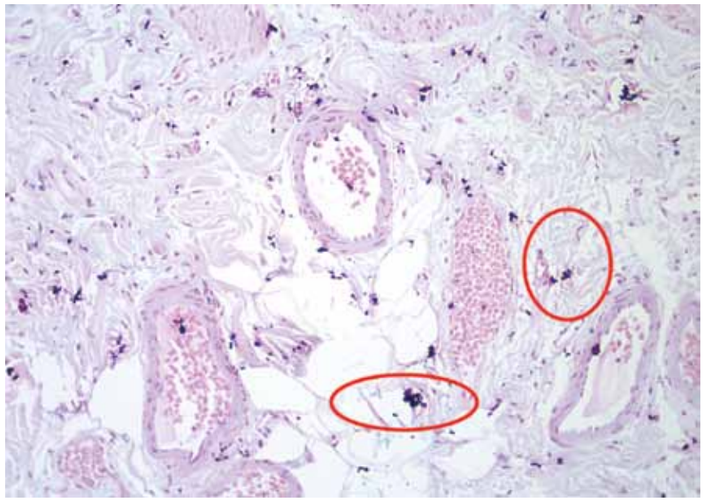

Figure 3a. Nuclear Fast Red precipitate.

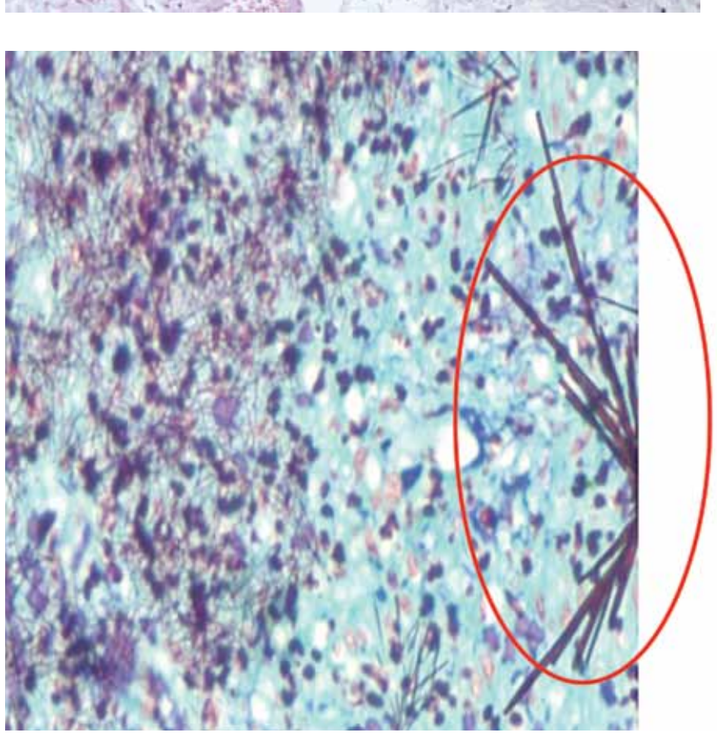

Figure 3b. Crystal Violet showing shards.

Reagents used on **Artisan**Link have been validated and optimized for staining quality as well as for correct storage and usage temperatures. It is important that the user follows the recommended temperature for storage and usage for each of the staining kits. All **Artisan**Link staining kits have been validated to be used at room temperature. Deviating from the recommendations may cause an uneven staining, shorten the shelf life, and possibly cause a false negative staining. Reagent storage and usage temperatures can be located on the reagent packs and in the package inserts.

Background Staining

Background staining is a common problem when using charged slides or when there are additives in the water bath or by heat. When using a methenamine silver solution, a “mirroring” also know as grey haze can occur on the slide. “Mirroring” is defined as metallic silver deposited non-specifically over large areas of the front and back of the glass slide. The silver solution deposits a fine, brown-black, granular precipitate over the section and the glass portion of the slide (Fig. 4). Microscopically, this type of precipitate is usually not found on the same plane as the tissue (1).

Figure 4. Examples of “mirroring”/grey haze. The cause of this effect is over-heating of the methenamine solution.

To prevent “mirroring” or grey haze, it is important to check the temperature of the slide heater and/or the incubation time set in the software. Using non-charged slides (except for decalcified tissue) can also help lessen the amount of background staining when performing stains with silver nitrate solutions.

Inconsistent Staining

This may be caused due to:

- Inconsistent tissue thicknesses from slide to slide. For instance, thicker sections will show a strong staining, whereas thinner sections will show a weak staining (Fig. 5). It is important to keep to the correct thickness throughout the microtomy sectioning to obtain a consistent staining.

Figure 5. Inconsistent tissue thicknesses from the same run. Warthin-Starry Stain.

- Cold reagent packs not brought to room temperature before use. All reagent packs used on **Artisan**Link Special Staining System must be brought to room temperature (22-25°C). Allow 45 minutes for the reagent packs to warm up before placing them on **Artisan**Link. Using a cold or cool reagent pack may prevent the chemical reaction from taking place, and will give the impression that the instrument is producing inconsistent staining results within the same run.
- Reagent packs not primed before use. This can cause either a non-dispense of reagent or an incomplete dispense of reagent. It is important to always prime the packs before daily use to ensure proper dispensing of reagents.
- Uneven staining. This can be caused by the reagent not spreading evenly across the tissue section or sections. This is particularly problematic with reagents containing acids such as acetic acid or periodic acid. The solution to this problem is to soak the slides in an Artisan™ Wash Solution for five minutes before loading the slides onto the slide carousel. This procedure conditions the slides for spreading the reagents (Fig. 6).

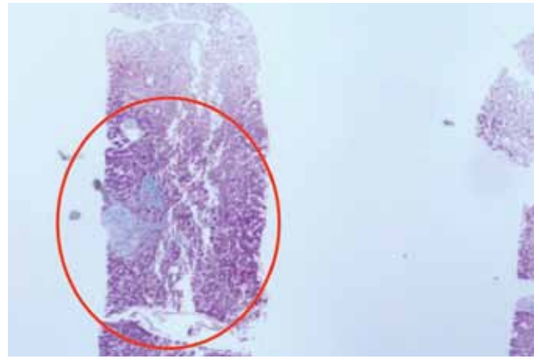

Figure 6. Periodic Acid-Schiff Diastase (m/D) staining of liver tissue. The uneven staining is due to amylase not spreading evenly- causing part of the liver section to stain undigested glycogen.

Another reason for an uneven staining may be the placement of the tissue sections on the slide close to the slide clip. This can be rectified by making sure that the tissue sections are centered on the slide. Placing the sections close to the slide clip causes a “wicking” effect which prevents complete aspiration of the reagents causing an uneven staining.

Issue	Manual Procedure	**Artisan**Link Procedure
Staining Artifact	Store reagents at correct temperature; (See package insert for specification)	Store reagent packs at correct temperature; (See package insert for specification)
	Filter reagents before use	Mix and prime reagents before use
	Use clean slides	Use clean slides
	Clean glassware in soap and hot water	Clean bulk liquid containers
	Rinse in deionized water and air dry	Check dispenser tip for debri build-up
Background Staining	Use acid clean glassware	Use the default protocol for silver stains
	Do not use metal forceps	Do not add adhesives to the water bath
Inconsistent Staining	Employ proper fixation, dehydration and infiltration	Employ proper fixation, dehydration and infiltration
	Increase or decrease staining time by looking at individual slides under the microscope	Cut all sections into a thickness of 4 µm, except for Jones' Basement Membrane Stain for kidney which should be cut into a thickness of 2 µm and Congo Red Stain which should be cut into a thickness of 8 µm
	For an uneven staining, adjust the volume of reagent used to ensure proper spreading	Soak the slides in Artisan™ Wash Solution for five minutes before placing them on the instrument

Conclusion

The solution to staining artifacts, background staining and inconsistent staining for both the manual and the automated procedures is very similar. The table above shows the comparison.

ArtisanLink Special Staining System provides the histotechnologist with a tool to produce the same diagnostic quality stains as found in a manual staining technique. However, unlike a manual staining, **Artisan**Link allows the histotechnologist to walk away from the special staining bench to pursue other tasks.

Reference

1. Brown RW (Ed.) (2009). Histologic Preparations: Common Problems and Their Solutions. College of American Pathologists, Northfield IL, 2009.